A PHYTOPHTHORA ROOT ROT OF SOYBEANS

DISSERTATION

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INTRODUCTION

A destructive root rot of soybeans was first observed in Sandusky County in Northwestern Ohio in 1951. The disease was reported in 1951 by the agricultural agent in this area. Subsequently, in the summers of 1953 and 1954, this root rot was observed in Allen, Auglaize, Erie, Ottawa, Paulding, Putnam, and Van Wert Counties. The disease was found generally distributed in the old lake-bed soils, such as Brookston, Clyde, and Toledo silty clays.

In 1953, losses in yield due to this disease were serious in many soybean fields. In 1954, the disease was recognized as of extreme economic importance in Northwestern Ohio. On the basis of histories of field crop diseases, this disease will likely be a limiting factor in the production of soybeans in Ohio.

As far as the writer is aware, this is the first report of this disease.

The present study was initiated to determine the causal agent, factors influencing its pathogenicity, and possible control measures.

REVIEW OF THE LITERATURE

A literature review of *Pythium* species as incitants of soybean diseases is included because the fungus used in this research was first identified as a species of *Pythium*. The incitant was later more accurately identified as a species of *Phytophthora*. In the literature, many *Pythiums* were not identified as to species. It is possible that the investigators worked with *Phytophthora* species. The means of distinguishing the 2 genera are not too definite especially if reproductive bodies are not readily found. The taxonomy of the *Pythium* and *Phytophthora* genera is largely based on morphological and physiological characteristics and the identification-keys are based on such factors as growth on certain media, type of antheridia, character of sporangia, temperature relations, pathogenicity, and development of certain types of reproductive organs. Since these characteristics are extremely variable, specific distinctions within and between the genera are limited.

In 1924, *Pythium debaryanum* Hesse was reported by Wolf and Lehman (25) as the causal agent of a root rot of soybeans in North Carolina. Two years later, these same authors described the symptoms of the disease and the morphology of the fungus (13). *Pythium ultimum* Trow and a number of other species of *Pythium* were eliminated as causal organisms on the basis that they were saprophytes. Since that time however, *P. ultimum* has been reported as parasitic to many plant species.

Hildebrand and Koch (7), in 1952, described a stem and root rot on a number of soybean varieties caused by P. ultimum. These investigators were of the opinion that the fungus described in Lehman and Wolf's paper (13) could be more accurately identified as P. ultimum.

According to Sprague (22), P. debaryanum was frequently isolated from soybean seed in western North Dakota. In 1946, McLaughlin (18) reported that P. debaryanum was the causal agent of a damping-off of soybeans in Oklahoma.

Porter (20), in 1946, found P. debaryanum as the causal organism of a so-called "baldhead" soybean seedlings, in which the plumules failed to develop.

Many investigators have reported unidentified species of Pythium in association with damping-off and seedling blight of soybeans (2, 23). According to Bretz (3), in 1944, a species of Pythium caused a wilting and drying of soybean plants. Species of Pythium were reported as causal agents of a root necrosis of soybeans (4, 10). In 1948, McNew (19), in Iowa, found a species of Pythium associated with a root and neck rot of soybeans. Koehler (11) reported a Pythium species as responsible for a root and stem rot. Ling (16), in 1948, included a species of Pythium in a list of 20 fungi parasitic to soybeans in China.

As far as the writer is aware, no species of Phytophthora have been reported as incitants of a root rot of soybeans.

METHODS AND MATERIALS

Inoculum:

Stock cultures of the Phytophthora species were maintained on yeast extract and corn meal agars at 20° C.

For infestation of the soil, Phytophthora was increased in a liquid medium containing broth from 100 grams of potatoes, 10 grams of dextrose, and distilled water to make 1 liter. One hundred ml of the broth in 250 ml Erlenmeyer flasks were autoclaved for 30 minutes at 15 pounds pressure. A small piece of mycelium from stock cultures was transferred to each flask; after 2 weeks at room temperature, the mycelium that developed was macerated in a Waring blendor for 30 seconds.

Unless otherwise specified, 100 ml of a 2-week old culture from potato-dextrose broth were evenly distributed over the soil in each 6-inch pot. The clay pots had been washed, then autoclaved for 8 hours at 20 pounds pressure, before use. The soybean seeds were planted, then covered with approximately 1 inch of soil. Checks were treated in a similar manner except that sterile potato-dextrose broth was used.

Soils:

Wooster silty loam was used in experiments on the effect of soil temperatures on the pathogenicity of the Phytophthora species. For other tests, several types of soil were used: loam-sand (1 part of sand and 2 parts of loam), sand, and Toledo silty clay obtained from Northwestern Ohio. All soils were autoclaved at 220° F. for 8 hours at 20 pounds pressure.

Sources of seed:

The soybean seeds used in greenhouse experiments and in the disease nursery at Oak Harbor, Ohio, were generously furnished by The Agronomy Department, The Ohio State University and The Northern Regional Soybean Laboratory at Urbana, Illinois, respectively. The seeds used in the suscept range experiment were supplied by the Livingston Seed Co. of Columbus, Ohio.

Except for the seed-treatments and field work, all soybean seeds were surface-sterilized, prior to planting, in a 1:1 solution of sodium hypochlorite.

Soil temperatures:

A series of Wisconsin-type temperature tanks (14) were used in experiments on the effect of soil temperatures on the pathogenicity of the Phytophthora species. Soil temperatures were maintained at 15°, 25°, and 35° C. The canisters and soil were autoclaved for 8 hours at 20 pounds pressure. One hundred and fifty ml of 2-week old inoculum in potato-dextrose broth were added to each 8-inch canister. Twenty-five seeds were planted in each canister. Water was added, throughout the experiment, to maintain the soil in a moderately moist condition.

Treatment of soybean seeds:

Soybean seeds were treated in units of 125. Each unit of Earlyana and Harosoy seeds weighed approximately 27 grams. The seeds were placed in extra-wide-mouth specimen bottles and chemicals were applied at the rate of 2 oz. per bushel. The seeds were well coated with the chemicals by rolling the bottles for

15 minutes on a machine similar to the one described by McCallan (17).

Inoculation of soybean transplants:

Two-week old plants, growing in sterilized white quartz sand, were carefully removed and washed thoroughly in tap water. The plants were inoculated by immersing and swirling the roots in an inoculum suspension and then transplanted to pots of soil. The remainder of the 100 ml portion of inoculum was poured onto the soil in each 6-inch pot.

Measurements of pH:

Measurements of pH were made with a Beckman meter, model H2. The glycine buffer systems used were those of Sorensen, prepared according to Gortner (6). A pH reading was made of each sample of buffered corn meal agar before it was poured into Petri plates.

Fertilizer treatment:

In the experiments on the effect of fertilizer on the pathogenicity of the Phytophthora species to soybeans, 1.14 and 2.28 grams of 0-20-20 fertilizer were added to 468 cubic inches of Toledo silty clay. This represented application rates of 200 and 400 pounds per acre, respectively. The soil was sifted several times through a $\frac{1}{4}$-inch mesh wire following uniform spreading of the fertilizer over the surface.

Growth of the fungus on various media:

Rate of growth in diameter of the Phytophthora colonies was obtained on several different media at temperatures ranging

from 6° to 35° C. Small pieces of mycelium and agar from stock cultures were transferred to various media in Petri plates. The compositions of the different media were:

Corn meal agar

distilled water	1000 ml
yellow corn meal	50 g
agar	15 g

Malt agar

distilled water	1000 ml
malt extract	15 g
agar	17 g

Yeast extract agar

distilled water	1000 ml
yeast extract	2.0 g
sodium nitrate	1.0 g
monobasic potassium phosphate	1.0 g
magnesium sulfate	0.5 g
trace-element stock solution[a]	1 ml

Difco potato-dextrose agar

Difco nutrient agar

The Difco agars were prepared according to the manufacturer's direction.

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[a] The trace-element stock solution consisted of:

distilled water	1000 ml
ferric ammonium citrate	0.9 g
zinc chloride	0.6 g
cupric chloride	0.2 g
manganese chloride	0.2 g

All media were autoclaved 20 minutes at 15 pounds pressure. Increase in the diameters of the fungus colonies per unit of time in millimeters was recorded. The experiment was repeated using 4 replicates of each treatment.

Soybean disease nursery:

In the summer of 1954, a soybean disease nursery was established at Oak Harbor, Ohio, on Toledo silty clay soil. Every soybean plant examined on this site in 1953 had symptoms of the root rot disease. Seven classes of 95 soybean varieties and strains were planted in a randomized block design with 2 replications of 4 different planting dates. The seeds were sown in 8-foot rows, 3 feet apart. Notes on disease incidence were taken on September 2 to 4.

Recording of data:

In greenhouse tests, healthy and diseased soybean seedlings were counted 3 weeks after the seeds were planted. The roots and stems of surviving plants were examined for internal discoloration. Percentage of diseased plants was calculated on the basis of the number of soybean seeds planted. An adjustment for non-viable seed was obtained by the difference between the number of seeds planted and the number of seedlings that emerged in the checks. The number of plants in the pre-emergence damping-off phase was the difference between the number that emerged in the checks and in the infested soil. The number of soybean plants in the post-emergence phase was the difference between the number that emerged in the infested soil and the number

that survived.

In all tests, the Phytophthora species was re-isolated from diseased tissue fragments which were plated on corn meal agar.

Statistical methods:

The complete randomization design was used in all experiments. Whenever the data fulfilled the assumptions of the analysis of variance, tests of significance were made by the F test (21). To obtain information on significant differences between individual means, the L.S.D. (least significant difference) as described by Johnson (9), was utilized.

Orthogonal sets of comparisons, as described by Johnson (9), were used in all instances where a comparison between two treatments was desired. In this paper, the terms significant and highly significant refer to differences which, due to their magnitude, can be expected to occur by chance alone in not more than 5 per cent and 1 per cent of the trials, respectively.

EXPERIMENTAL RESULTS

Symptoms

In the field, attention is first attracted to this soybean disease by the presence of scattered groups of stunted, wilting, and dying plants. Many small soybean seedlings are dry, brown, and contrast sharply with the surrounding healthy plants (Figure 1). Figure 2 is the general appearance of a representative field of soybeans in which the Phytophthora root rot is severe. Pre-emergence damping-off and seed decay, post-emergence damping-off, and root rotting associated with varying degrees of foliage chlorosis and wilting, are 3 phases of this disease.

Pre-emergence damping-off and seed decay:

Pre-emergence damping-off and seed decay is one phase of this soybean disease. In the field, large gaps in rows of soybeans may be due to pre-emergence damping-off or seed decay. In the greenhouse, pre-emergence damping-off is evident by fewer seedlings in infested soil than in non-infested soil.

Post-emergence damping-off:

The post-emergence damping-off phase of the disease results in the death of seedlings before the first trifoliate leaf expands. The hypocotyl and root system are water-soaked and brown. Frequently, portions of the emerged cotyledons are water-soaked and discolored (Figure 3). Collapse of the hypocotyl is followed by desiccation of the seedling in 2 to 4 days. The seedling shrinks to an inconspicuous brown, slender filament. The

rapidity of disease development and desiccation may account for the failure to recognize the early phase of the disease in the field. Young, diseased seedlings are best to use in isolating the pathogen. If older plants are used, considerable difficulty is encountered because of the rapid growth of secondary organisms, especially species of Fusarium.

Root rotting and wilting:

Root rotting and wilting are symptoms of those plants that survive the early seedling stages of disease. The first symptom is marked stunting (Figure 4). Wilting and chlorosis of the foliage are prominent symptoms also. Wilting may be sudden or prolonged over a period of 2 to 3 days. Patches of yellow tissue are evident at the periphery of the leaf and between the veins. Gradually, the entire leaf becomes chlorotic, dry, and brittle but does not abscise. This series of events in the root rotting phase of the disease is illustrated in Figures 5 to 7.

A dark-brown discoloration of the vascular tissues as well as an extensive decay of the root system is apparent in the stem and root of a diseased plant. The extent and intensity of vascular browning varies with variety. Discoloration is often found in the second and third nodes. Most of the secondary roots as well as the tap root are destroyed (Figures 8 and 9). The roots are usually so severely decayed that most break when plants are pulled from the soil. Soybean plants that are stunted, but otherwise healthy, frequently have an internal vascular discoloration.

Figure 1.--Seedling wilt of Hawkeye soybean plants incited by a Phytophthora species. Healthy plants on right.

Figure 2.--Field of Bavender soybeans in which *Phytophthora* root rot is severe.

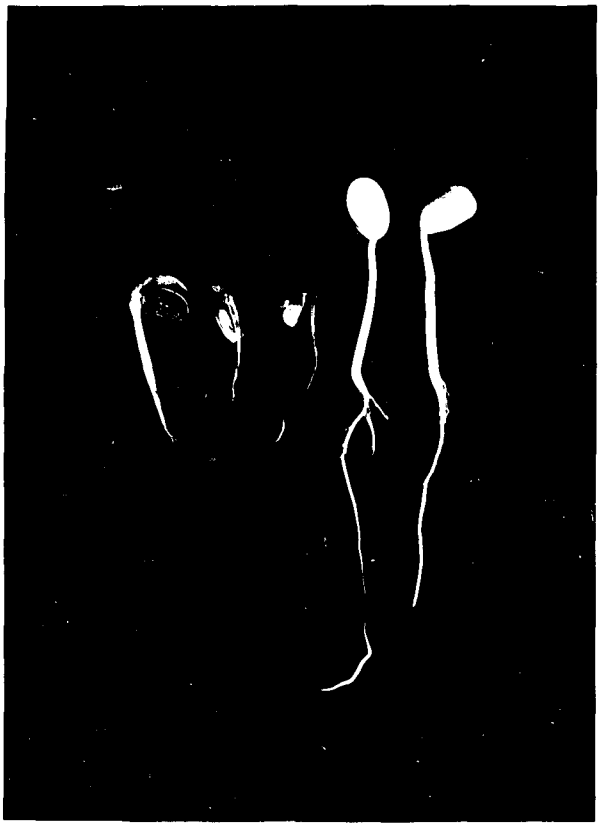

Figure 3.--Diseased Earlyana soybean seedlings with cotyledons partially water-soaked and discolored. Two healthy seedlings on right.

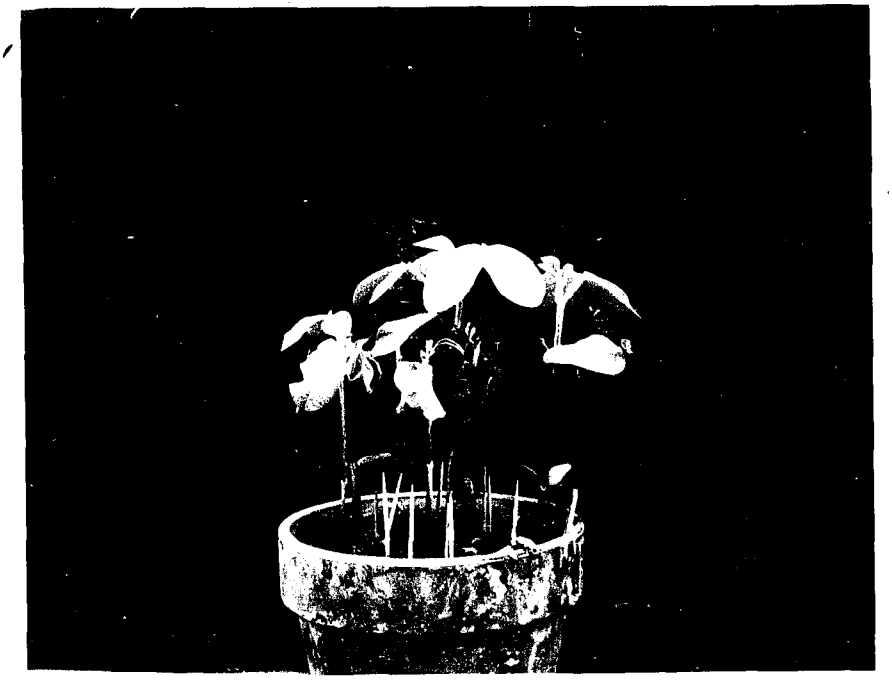

Figure 4.--Harosoy soybean plants in Phytophthora-infested soil. Note stunted plants with flaccid unifoliate leaves not yet chlorotic. Toothpicks designate seedlings that died soon after emergence.

Isolation of the Fungus

Diseased soybean plants were collected in 1953 in Northwestern Ohio at regular intervals beginning when the plants were 3 weeks old. Fragments of internal stem and root tissue were excised and transferred to plates of potato-dextrose and yeast extract agars. As soon as fungus growth appeared, small pieces of the mycelium and agar from the periphery of a developing colony were transferred to tubed slants of 2 per cent potato-dextrose agar. Isolates arising from approximately 600 tissue platings consisted of Fusarium species, 72 per cent; Rhizoctonia species, 10 per cent; Gliocladium species, 10 per cent; and other species, 8 per cent. The pathogenicity of representative isolates from each of the groups was tested and none was pathogenic to soybean plants.

During the summer of 1954, collections of diseased plants were started a week earlier than in 1953. A species of Phytophthora was frequently isolated from several lots of diseased, 2-week old soybean seedlings. Diseased tissue fragments were washed in tap water, then immersed in sterile water and placed in tubes, each containing 20 cc of sterile water.

After 24 hours at room temperature, the tubes were examined for mycelial growth surrounding the pieces of diseased tissue. The tissue fragments were then removed from the tubes and blotted between two pieces of filter paper to remove the excess water, and with it, a large number of bacterial contaminants. After blotting thoroughly, the tissue fragments were transferred

Figure 5.--Hawkeye soybean plants in the early wilt phase of the Phytophthora root rot. No chlorosis of the foliage is evident in this stage. Healthy plants left of center.

Figure 6.--Hawkeye soybean plants in the wilt-chlorotic phase of the Phytophthora root rot. The affected leaves are brittle. Healthy plants on right.

Figure 7.--Hawkeye soybean plants in the final stage of the Phytophthora root rot. The diseased plants are necrotic and dry. Plant on right is stunted. Healthy plants on left.

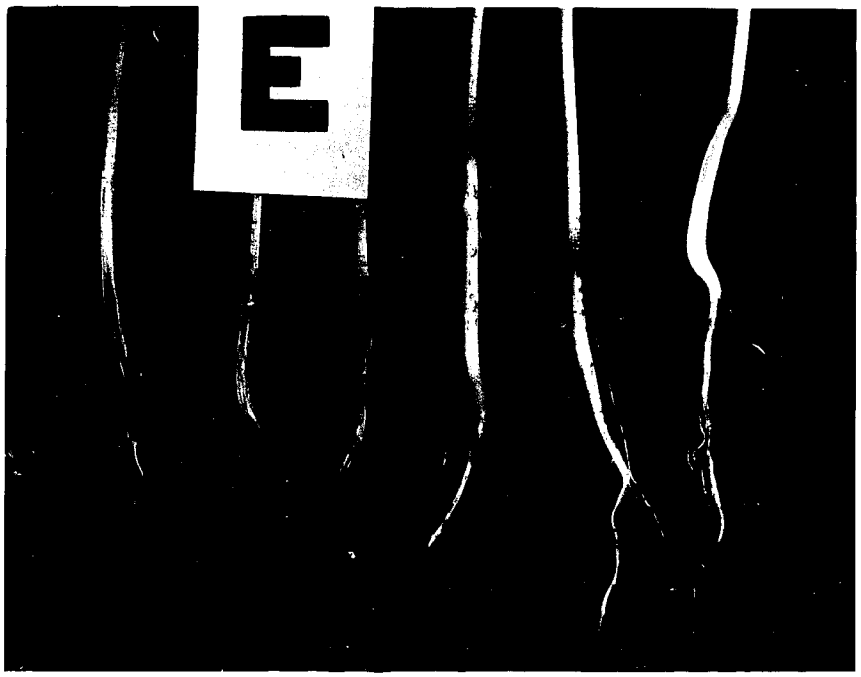

Figure 8.--Root systems of 3-week old Earlyana soybean plants from Toledo silty clay. Three plants on left from Phytophthora-infested soil; others from non-infested soil.

Figure 9.--Three-week old soybean plants of the Earlyana variety. Note the extensive decay of the roots of the 3 plants on right. Two healthy plants on left from steamed, non-infested soil.

to corn meal agar. Isolates of the fungus were obtained also by transferring tissue fragments from diseased soybean seedlings to plates of corn meal agar. Species of Phytophthora were isolated from 87 per cent of a total of 60 tissue transfers. These isolates, macroscopically at least, were identical and similar in pathogenicity on Bavender variety of soybeans. One isolate was selected at random and used in all subsequent experiments.

The species of Phytophthora isolated is slow-growing which may explain why it was not isolated previously on potato-dextrose and yeast extract agars. Bacteria were associated usually with the Phytophthora cultures. Fungus colonies free of bacteria were obtained by the method described by Ark and Dickey (1). Three plastic modeling clay pellets are attached to one end of a Van Tieghem cell. The cell is then centrally placed in the bottom of a Petri dish, with the pellets against the glass. The cell is 2-3 mm from the bottom of the Petri dish. The Petri dish cover is replaced and the entire apparatus is autoclaved. After sterilization, a thick layer of warm 3 per cent agar medium is poured into the Petri dish. A fragment of diseased tissue is rinsed in sterile water and then placed on agar within the cell. The fungus hyphae grow into the agar and emerge outside of the Van Tieghem cell free of bacteria.

Habitat of the Pathogen

Soil samples were obtained from several fields in which specimens of diseased plants were periodically collected. A portion

of each of the samples was autoclaved for 8 hours at 20 pounds pressure, and placed in clay pots which were previously autoclaved. The other portion of non-steamed soil of each sample was placed in another set of pots. Twenty seeds of the Bavender variety were sown in each pot. Symptoms of the disease developed in most of the plants in the non-steamed soil. Phytophthora was isolated from these plants. None of the plants in the steamed soil was diseased. It was inferred that the causal agent was in the soil.

Seeds of variety Bavender, and strains W9-2024 and AX63-129-1-2 were selected at random from the same seed lot and immersed for 2 minutes in a 1:1 solution of sodium hypochlorite. After several rinses in sterile water, the seeds were placed in Petri dishes containing corn meal and 2 per cent water agars and incubated at 20° c. A similar set of dishes, containing seeds which were not surface-sterilized, was included. Phytophthora did not grow from any of the soybean seeds. Species of Cercospora, Alternaria, Penicillium, and Rhizopus were isolated from some of the seeds.

The occurrence of diseased soybean plants scattered throughout the field, is indicative of the distribution pattern of soil-borne pathogens. In Ohio, this disease has been found only in fields of soybeans growing in clay and silty clay soils, such as Brookston, Clyde, and Toledo silty clays. The disease is usually more severe in poorly drained sections of the field.

The Pathogen

Morphology

The young mycelium of the Phytophthora species, pathogenic to soybean plants, is coenocytic, non-septate, and branches freely. In older cultures, the mycelium is septate and 2.4 to 5.0 microns in diameter. The hyphae are tuberculate on corn meal and potato-dextrose agars.

Sporangia are ovoid to ellipsoid and are non-papillate. Sporangia, in diseased soybean tissues, range from 12 to 15 microns in width and from 23 to 25 microns in length. Formation of zoospores was not induced by flooding or cooling the fungus colonies.

Oogonia are globose and 20.6 to 25.4 microns in diameter. The walls of the oogonia are thin and smooth. Oogonia were not observed on potato-dextrose, malt, or nutrient agars. Oogonia form abundantly on corn meal agar and on grated carrot agar prepared according to Johann (8).

Oospores are smooth, globose, and vary from 10 to 15 microns in diameter. The walls of the oospores are smooth and about 1.3 microns in thickness.

Antheridia are amphigynous, i.e., envelope the oogonial stalk.

Identification

Final identification of the Phycomyceteous pathogen was made by Dr. John T. Middleton, Citrus Experiment Station,

Riverside, California, from a culture sent him.

Growth on different media

The appearance of the Phytophthora species on various media is illustrated in Figure 10. On each of the 5 media, the colony and growth characteristics are strikingly different. On corn meal agar, the white mycelium is delicate and scarcely visible unless examined by transmitted light. The mycelium is chiefly intramatrical with limited aerial growth. The mycelial growth is white and dense on potato-dextrose, malt, and yeast extract agars. The growth habit on the former medium is cumulous. On yeast extract agar, the growth habit is pulvinate, i.e., cushion-shaped or strongly convexed. The delicate mycelium on nutrient agar is chiefly intramatrical and tan.

Growth-temperature relations

The effects of temperature on radial growth of the Phytophthora species were studied. The fungus was transferred to 5 different media in Petri dishes and incubated at various temperatures ranging from 6° to 35° C. The temperatures were kept constant by controlled incubators. Data were recorded on the diameters of colonies at 2-day intervals. The average diameter data of the Phytophthora colonies on different media after 8 days are illustrated in Figure 11.

The rate of growth of the Phytophthora species was greater on corn meal agar than on nutrient, potato-dextrose, yeast extract, and malt agars, respectively. The cardinal temperatures for growth on corn meal agar were: less than 6°, 26°, and greater

Figure 10.--Growth of the Phytophthora species on various media after 19 days incubation at 20° C. Top row, left to right: malt, yeast extract, and potato-dextrose agars. Bottom row, left to right: nutrient, and corn meal agars.

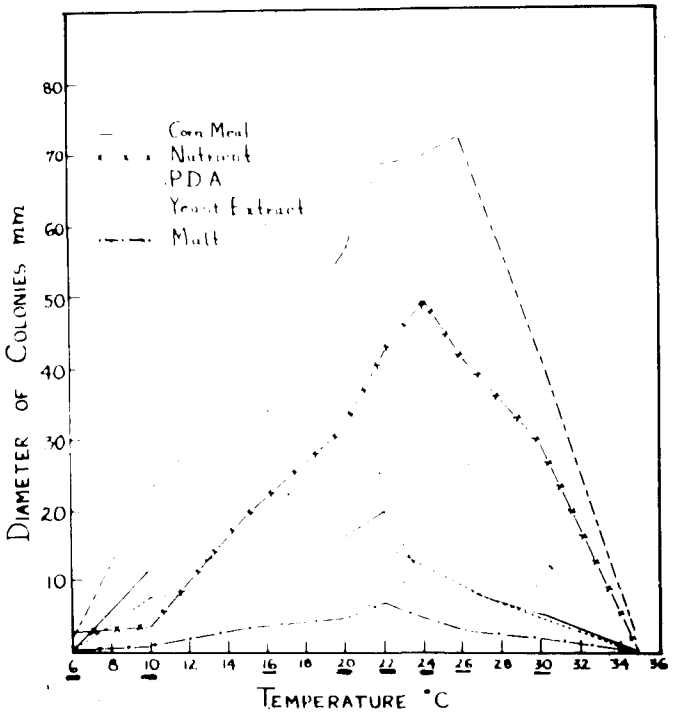

Figure 11.--Diameter of Phytophthora colonies after 8 days at different temperatures on various media.

than 35° C., respectively. On nutrient agar, the respective cardinal temperatures were: less than 6°, 24°, and 30° C. The cardinal temperatures for growth of the Phytophthora species on potato-dextrose, malt, and yeast extract agars were: 10°, 22°, and 30° C., respectively.

Effect of pH on growth

Several lots of corn meal agar were adjusted to different degrees of acidity and alkalinity to study the effect of hydrogen-ion concentration on the growth of the Phytophthora species. MacIlvain's buffer mixtures of citric acid-disodium phosphate were used for the range pH 2-8 (12). These mixtures were apparently toxic to the fungus as no growth occurred in this range. Sorensen's buffer mixtures of hydrochloric acid-glycine were substituted (6). For the range pH 9-11, mixtures of sodium hydroxide-glycine were prepared according to the descriptions by Gortner (6). A pH reading was made of each sample of agar before pouring the plates. Four Petri plates of agar were used for each pH value and incubated at 26° C. The average diameter data of the Phytophthora colonies at various hydrogen-ion concentrations is presented in Figure 12. The optimum pH for growth was 9, with a secondary optimum at approximately pH 6.3.

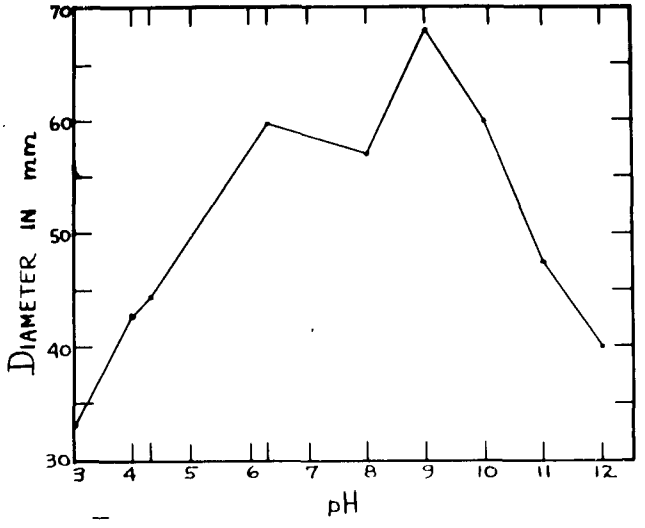

Figure 12.--Diameter of Phytophthora colonies after 8 days at different hydrogen-ion concentrations on buffered corn meal agar.

Field Test in 1954

In the summer of 1954, a plot at Oak Harbor, Ohio, was selected for a soybean disease nursery. This area included a portion of a 25-acre field in which nearly all Bavender soybean plants, examined during 1953, had been diseased. Seven classes of soybean varieties and strains, arranged in 5 maturity groups, were planted. In general, each group included soybean strains differing by not more than 10 to 15 days in maturity date. Group 0 contained strains that bloom and mature under the longer days encountered during the summer in the Dakotas, Minnesota, and northern Wisconsin. Group I consisted of strains generally cultivated in South Dakota, the southern parts of Minnesota, Wisconsin, Michigan, and the northern part of Ohio. Groups II, III, and IV, respectively, included strains which are common to the locations farther south in the North Central States and to areas of similar latitude. State Varieties and the Advanced Preliminary classes included new strains, with desirable agronomic characteristics, that are distributed among the maturity Groups I and II.

Using a randomized block design, each variety or strain was replicated twice for each planting date. The 4 planting dates were: May 26, June 2, June 9, and June 22, 1954. Because of the extremely dry weather, seedling emergence after the first planting was poor and erratic.

The total number of plants that emerged in each 8-foot row was tabulated. The following categories of post-emergence disease incidence were assigned: seedling wilt, late wilt, internal browning, and total disease. The percentage of diseased plants was calculated on the basis of the total number of soybean plants that emerged in 8 replicates. In obtaining data on internal browning, 10 plants were selected at random from each row. The root and basal portion of the stem of each plant was examined.

Since the field data did not fulfill certain assumptions underlying the analysis of variance test, mainly, homogeneity of variance, they were not subjected to statistical analysis. Consequently, indices were assigned for each disease category. An index scale, representing the per cent of soybean plants diseased, was established as follows: 1, 0 to 20; 2, 21 to 40; 3, 41 to 60; 4, 61 to 80; and 5, 81 to 100. In evaluating the field results, emphasis was placed on the total disease incidence. Any variety or strain with an index of 1 (0 to 20 per cent of the plants diseased) was considered resistant.

The results of the field test are summarized in Tables 1 to 7. In maturity Groups 0, II, and IV, no soybean varieties or strains were assigned a total disease index of 1. Members of these maturity groups with low percentage of total disease incidence warrant further consideration as breeding material.

A total disease index of 1 was assigned to Blackhawk, Monroe, and strain C1109 of maturity Group I. Soybean strain W9-2024, used throughout the greenhouse experimentation, was

assigned a total disease index of 5, and the incidence of seedling wilt was high as evidenced by an index of 3. All varieties of Group I had an index of 1 for seedling and late wilt, whereas the indices for internal browning were high. Earlyana and strains C1112, C1117, and M-13 each had a total disease index of 3 chiefly because of severe internal discoloration.

Adams and strain H13501, with a total disease percentage of 24.7 and 27.1 respectively, were the best members of maturity Group II. Except for the variety Harosoy, all soybean varieties and strains of Group II were assigned an index of 1 for seedling and late wilt.

All members of the Advanced Preliminary Group, except strain 24088, were assigned an index of 1 for seedling and late wilt. Soybean strains 24338, 22218, 22153, 24157, and OH-53 of this Group each had a total disease index of 1. An index of 4 for internal browning was assigned to strain 14775.

All members, except strain 24050, of the State Variety Group had indices of 1 for seedling and late wilt. Soybean strain 24050 was assigned an index of 2 for seedling wilt, but an index of 5 for total disease. Strain 15368 with a total disease index of 2 and percentage total disease incidence of 37.1 was the best member of the State Variety Group.

Illini with an index of 1 for all disease categories was the only resistant variety of Group III. The other members of this maturity group were assigned an index of 1 for seedling

and late wilt. The incidence of internal browning in these strains and varieties was severe, however.

No members of Group IV were assigned total disease indices less than 3 (41 to 60 per cent of the plants diseased). Only strains C1076 and C1079 were assigned indices of 3. In regard to seedling and late wilt, all soybean strains had an index of 1. As in maturity Group III, the incidence of internal browning was severe in this Group.

Comet, Renville, and strain WOS-3180 of Group 0 with percentage total disease of 28.1, 30.6, and 33.8, respectively were assigned a total disease index of 2. Soybean strain WOS-3138 was severely affected in the early phase of disease development as evidenced by an index of 3 for seedling wilt. All members of this maturity group were assigned an index of 1 for the incidence of late wilt.

No relationship between the pedigree of soybean strains and susceptibility to the root rot was apparent. Blackhawk and Hawkeye are different selections from the same cross (Mukden x Richland), but Hawkeye was susceptible whereas Blackhawk was resistant.

From the field results, it was inferred that time of planting had no effect on disease incidence. No difference in susceptibility was evident in any soybean variety or strain at any of the 4 planting dates.

Table 1.--Post-emergence disease development of soybeans of maturity Group I in naturally-infested Toledo silty clay at Oak Harbor, Ohio, in 1954.

Strain or Variety	Origin	Percentage of plants in each disease category[a]			
		Seedling Wilt	Late Wilt	Internal Browning	Total
Blackhawk	Sel. from Mukden x Richland	1[b]	1	1	1
Earlyana	Sel. from a natural hybrid	1	1	3	3
Monroe	Sel. from Mukden x Mandarin	1	1	1	1
C 1105	Sel. from A4-107-12 x Man.(Ott.)	1	1	2	2
C 1106	do	1	1	2	3
C 1109	Sel. from Mukden x Man. (Ott.)	1	1	1	1
C 1112	Sel. from Mandarin (Ott.) x Lincoln	1	1	3	3
C 1117	do	1	1	3	3
C 1119	do	1	1	2	3
C 1121	do	1	1	2	2
H-10042	Sel. from Lincoln x (Richland x C11)	1	1	3	4
M-12	Sel. from Hawkeye x Flambeau	1	1	2	2
M-13	Sel. from Hawkeye x Ontario	1	1	3	3
W9-1486	Sel. from Hawkeye x Flambeau	2	1	2	4
W9-2024	do	3	1	2	5
AOK-2206	Sel. from Hawkeye x Man. (Ott.)	1	1	3	4
AOK-3808	Sel. from Lin. x (Lin. x Richland)	1	1	2	4

[a] Based on total number of plants that emerged in 8 replicates.

[b] Scale: 1 = 0 - 20% plants diseased, 2 = 21 - 40% plants diseased, 3 = 41 - 60% plants diseased, 4 = 61 - 80% plants diseased, 5 = 81 - 100% plants diseased.

Table 2.--Post-emergence disease development of soybeans of maturity Group II in naturally-infested Toledo silty clay at Oak Harbor, Ohio, in 1954.

Strain or Variety	Origin	Percentage of plants in each disease category[a]			
		Seedling Wilt	Late Wilt	Internal Browning	Total
Adams	Sel. from Illini x Dunfield	1[b]	1	2	2
Harosoy	Sel. from Man. x (Man. x A.K.)	2	1	3	4
Hawkeye	Sel. from Mukden x Richland	1	1	3	4
Lincoln	Sel. from Mandarin x Manchu	1	1	3	4
Richland	Sel. from P.I. 70502-2	1	1	3	3
AO-8618	Sel. from Lin. x (Lin. x Richland)	1	1	3	3
AX-29-163-1-2	Sel. from Adams x Hawkeye	1	1	3	5
C 1056	Sel. from Lin. x (Lin. x A45-251)	1	1	2	3
C 1128	Sel. from Wabash x A4-107-12	1	1	2	3
H 13116	Sel. from Lin. x (Richland x C11)	1	1	2	2
H 13501	do	1	1	2	2
H 14025	Sel. from Lincoln x Quebec 92	1	1	3	4
H 14521	Sel. from Lincoln x Ontario	1	1	3	4
H 15548	Sel. from Lincoln x P.I. 68666	1	1	4	4
L9-5139	Sel. from Lin. x (Lin. x Richland)	1	1	2	3

[a] Based on total number of plants that emerged in 8 replicates.

[b] Scale: 1 = 0 - 20% plants diseased, 2 = 21 - 40% plants diseased, 3 = 41 - 60% plants diseased, 4 = 61 - 80% plants diseased, 5 = 81 - 100% plants diseased.

Table 3.--Post-Emergence disease development of soybeans of the Advanced Preliminary Group in naturally-infested Toledo silty clay at Oak Harbor, Ohio, in 1954.

Strain or Variety	Origin	Percentage of plants in each disease category[a]			
		Seedling Wilt	Late Wilt	Internal Browning	Total
14574	L x 1044 Lincoln x Ontario	1[b]	1	3	4
20867	H x 2 Monroe x Lincoln	1	1	3	4
24338	H x 6 Richland x H-2	1	1	1	1
15375	H x 1 x Lincoln x P.I. 68666	1	1	3	3
22057	H x 9 H-5 x A 4-107-12	1	1	3	4
14775	L x 1044 Lincoln x Ontario	1	1	4	4
22218	H x 9 H-5 x A 4-107-12	1	1	1	1
24088	H x 2 Monroe x Lincoln	1	2	3	5
22547	H x 10 H-2 x A 4-107-12	1	1	2	2
15809	H x 1 x Lincoln x P.I. 68666	1	1	2	2
14692	L x 1044 Lincoln x Ontario	1	1	3	3
15255	L x 1043 Lincoln x P.I. 68666	1	1	3	3
22153	H x 9 H-5 x A 4-107-12	1	1	1	1
24157	H x 2 Monroe x Lincoln	1	1	1	1
OH-53	Sel. from Bavender Special	1	1	1	1

[a] Based on total number of plants that emerged in 8 replicates.

[b] Scale: 1 = 0 - 20% plants diseased, 2 = 21 - 40% plants diseased, 3 = 41 - 60% plants diseased, 4 = 61 - 80% plants diseased, 5 = 81 - 100% plants diseased.

Table 4.--Post-emergence disease development of soybeans of the State Variety Group in naturally-infested Toledo silty clay at Oak Harbor, Ohio, in 1954.

Strain or Variety	Origin	Percentage of plants in each disease category[a]			
		Seedling Wilt	Late Wilt	Internal Browning	Total
14597	L x 1044 Lincoln x Ontario	1[b]	1	4	4
15345	L x 1043 Lincoln x P.I. 68666	1	1	3	3
14551	L x 1044 Lincoln x Ontario	1	1	3	3
8178	Lincoln (Lincoln x Richland)	1	1	3	4
15368	H x 1 Lincoln x P.I. 68666	1	1	2	2
15518	do	1	1	3	3
15832	do	1	1	3	4
24050	H x 2 Monroe x Lincoln	2	1	2	5
20689	do	1	1	3	3
15285	L x 1043 Lincoln x P.I. 68666	1	1	3	4
15225	do	1	1	3	4
Mingo	Sel. from Manchu	1	1	3	4

[a] Based on total number of plants that emerged in 8 replicates.

[b] Scale: 1 = 0 - 20% plants diseased, 2 = 21 - 40% plants diseased, 3 = 41 - 60% plants diseased, 4 = 61 - 80% plants diseased, 5 = 81 - 100% plants diseased.

Table 5.--Post-emergence disease development of soybeans of maturity Group III in naturally-infested Toledo silty clay at Oak Harbor, Ohio, in 1954.

Strain or Variety	Origin	Percentage of plants in each disease category[a]			
		Seedling Wilt	Late Wilt	Internal Browning	Total
Illini	Sel. from A. K.	1[b]	1	1	1
Dunfield	Sel. from P. I. 36846	1	1	3	3
C 859	Sel. from Dunfield x Lincoln	1	1	3	3
U0 - 41	Sel. from U9 - 2	1	1	4	4
U9 - 2	Sel. from mixed seed	1	1	3	4
Clark	Sel. from Lin. x (Lin. x Richland)	1	1	4	4
C 1060	Sel. from Lin. x (Lin. x A 45-251)	1	1	4	4
I9-5139	Sel. from Lin. x (Lin. x Richland)	1	1	4	4

[a] Based on total number of plants that emerged in 8 replicates.

[b] Scale: 1 = 0 - 20% plants diseased, 2 = 21 - 40% plants diseased, 3 = 41 - 60% plants diseased, 4 = 61 - 80% plants diseased, 5 = 81 - 100% plants diseased.

Table 6.--Post-emergence disease development of soybeans of maturity Group IV in naturally-infested Toledo silty clay at Oak Harbor, Ohio, in 1954.

Strain or Variety	Origin	Percentage of plants in each disease category[a]			
		Seedling Wilt	Late Wilt	Internal Browning	Total
Chief	Sel. from Illini x Manchu	1[b]	1	3	4
Perry	Sel. from Patoka x L7-1355	1	1	4	4
Wabash	Sel. from Dunfield x Mansoy	1	1	3	4
C 985	Sel. from Lincoln x Ogden	1	1	3	4
C 1048	Sel. from Lin. x (Dun. x A45-251)	1	1	4	5
C 1065	Sel. from Lincoln x Ogden	1	1	3	4
C 1068	do	1	1	3	4
C 1069	do	1	1	4	4
C 1071	do	1	1	4	4
C 1074	do	1	1	4	4
C 1076	do	1	1	3	3
C 1078	do	1	1	4	4
C 1079	do	1	1	3	3

[a] Based on total number of plants that emerged in 8 replicates.

[b] Scale: 1 = 0 - 20% plants diseased, 2 = 21 - 40% plants diseased, 3 = 41 - 60% plants diseased, 4 = 61 - 80% plants diseased, 5 = 81 - 100% plants diseased.

Table 7.--Post-emergence disease development of soybeans of maturity Group 0 in naturally-infested Toledo silty clay at Oak Harbor, Ohio, in 1954.

Strain or Variety	Origin	Percentage of plants in each disease category[a]			
		Seedling Wilt	Late Wilt	Internal Browning	Total
Capital	Sel. from Strain 171 x A.K. (Harrow)	2[b]	1	2	5
Comet	Sel. from Pagoda x Mandarin	1	1	2	2
Flambeau	Sel. from Intr. from Russia	1	1	2	2
Hardome	Sel. from Mandarin x (Man. x A.K.)	1	1	3	3
Mandarin(Ott.)	Sel. from Mandarin	1	1	3	3
Renville	Sel. from Lincoln x (Lin. x Richland)	1	1	2	2
L6-6275	do	1	1	3	3
W6S-292	Sel. from Lincoln x Seneca	1	1	3	4
W8S-1460	Sel. from Hawkeye x Flambeau	1	1	3	3
W9S-2703	Sel. from Lincoln x Flambeau	1	1	2	3
WOS-3138	Sel. from Hawkeye x Flambeau	3	1	1	4
WOS-3147	Sel. from Mukden x Flambeau	1	1	3	3
WOS-3180	Sel. from Mukden x Flambeau	1	1	1	2
WOS-3257	do	1	1	2	3
WOS-3386	Sel. from Lincoln x Flambeau	1	1	3	3

[a] Based on total number of plants that emerged in 8 replicates.

[b] Scale: 1 = 0 - 20% plants diseased, 2 = 21 - 40% plants diseased, 3 = 41 - 60% plants diseased, 4= 61 - 80% plants diseased, 5 = 81 - 100% plants diseased.

Greenhouse Experiments on the Effect of Various Factors on Pathogenicity

Soil types

Since this root rot of soybeans has been found in Ohio only in clay and silty clay soils, the pathogenicity of the *Phytophthora* species in different soils was investigated. Toledo silty clay, loam, and sand were used. Three varieties of maturity Group I, Monroe, Earlyana, and W9-2024, in which the disease incidence ranged from high to low, were selected (see Table 1).

After the seedlings had emerged from the *Phytophthora*-infested series of soils, they were examined daily. Because of the rapid desiccation of the seedlings affected in post-emergence damping-off, some method of marking diseased seedlings was necessary. When the hypocotyls of seedlings began to turn brown, toothpicks were inserted in the soil adjacent to the seedlings.

The data are presented in Table 8, and the results are illustrated in Figures 13 to 15. The differences between the varieties and the soils were significant. Using the method of orthogonal comparisons, significant individual differences were obtained and are presented in Table 9.

Monroe was significantly more resistant than either Earlyana or W9-2024 in clay, loam, and sand (Figures 16 and 17). Monroe was significantly more susceptible in either loam or sand than in Toledo silty clay. Earlyana was significantly more susceptible in clay than in loam whereas strain W9-2024 was more susceptible in clay than in either loam or sand.

Table 8.--Pre-emergence damping-off (Pre), post-emergence damping-off (Post), and total disease incidence (Total) in soybean plants after 3 weeks in 3 types of soils infested with Phytophthora.

	Per cent of the plants diseased								
	Toledo clay			Loam			Sand		
Variety	Pre	Post	Total	Pre	Post	Total	Pre	Post	Total
Monroe	8.9	0.7	9.6	20.0	0.7	20.7	16.7	5.3	22.0
Earlyana	82.0	11.3	93.3	44.0	39.3	83.3	64.7	24.7	89.4
W9-2024	81.3	18.7	100.0	60.0	34.0	94.0	76.7	18.0	94.7

Table 9.--Significances of one-degree-of-freedom comparisons between the treatments of the data of Table 8.

	Toledo clay			Loam			Sand		
Treatment	Pre	Post	Total	Pre	Post	Total	Pre	Post	Total
Mon.-Early.	1[a]	1	1	5	1	1	1	1	1
Mon.-W9	1	1	1	1	1	1	1	5	1
Early.-W9	ns	ns	1	ns	ns	5	ns	ns	ns
	Monroe			Earlyana			W9-2024		
Clay-loam	1	ns	5	1	1	1	5	ns	1
Clay-sand	ns	1	1	5	ns	ns	ns	ns	1
Loam-sand	ns	1	ns	5	ns	ns	ns	ns	ns

[a] 1-1 per cent level of significance
5-5 per cent level of significance
ns - not significant

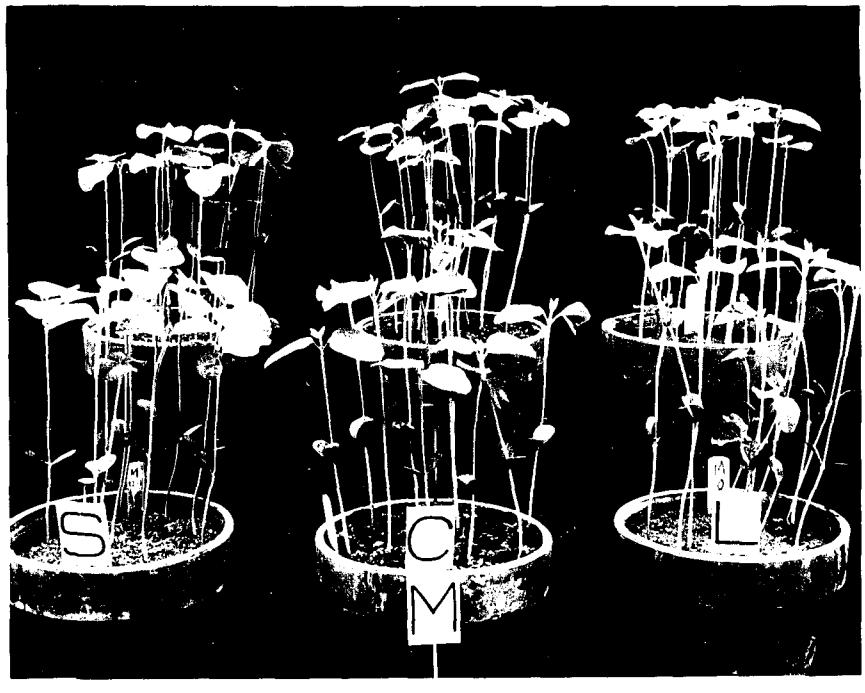

Figure 13.--Two-week old Monroe soybean plants in sand (S), Toledo silty clay (C), and loam (L). Back row, non-infested soil; front row, Phytophthora-infested soil.

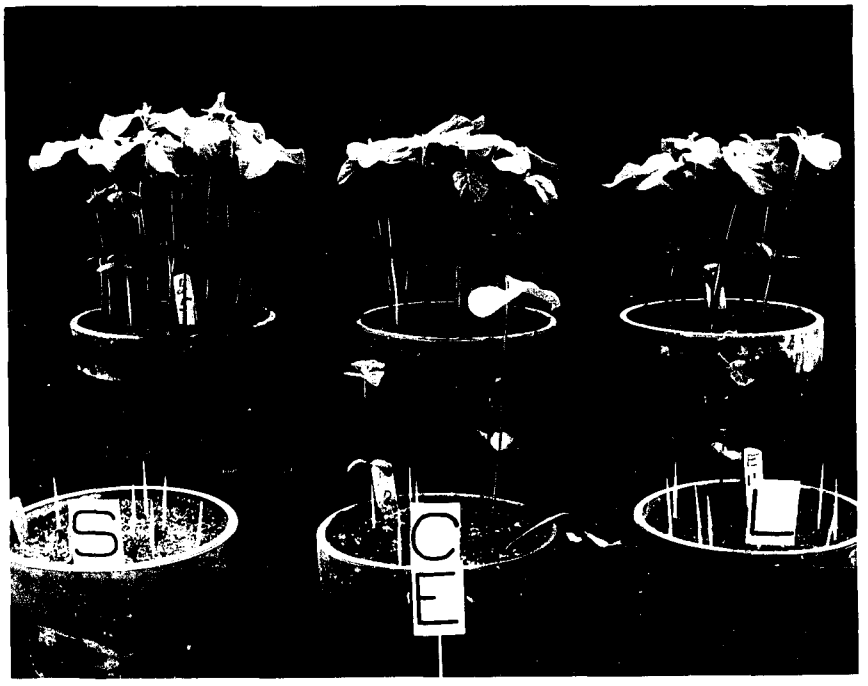

Figure 14.--Two-week old Earlyana soybean plants in sand (S), Toledo clay (C), and loam (L). Back row, non-infested soil; front row, Phytophthora-infested soil.

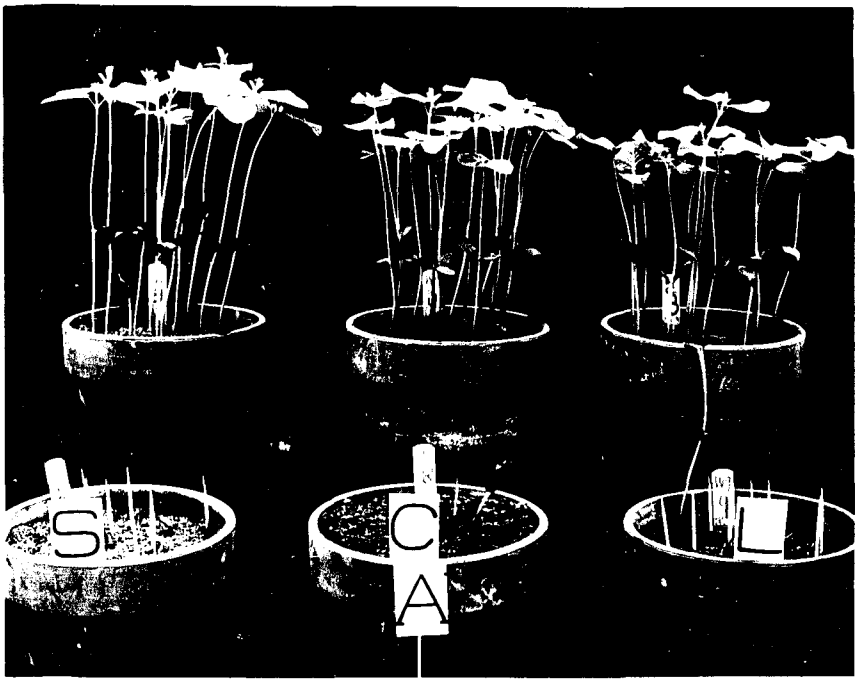

Figure 15.--Two-week old soybean plants of strain W9-2024 in sand (S), Toledo clay (C), and loam (L). Back row, non-infested soil; front row, Phytophthora-infested soil.

Relationship of age of plants and susceptibility

The relationship of the age of the soybean plants and susceptibility to the Phytophthora species was studied. Seeds of Monroe, Earlyana, and strain W9-2024 were sown in sterilized white quartz sand. When the seedlings were in the early, second true-leaf stage, they were transplanted to pots of loam infested with the Phytophthora species. Soybean seedlings affected in the early cotyledonary stage and those with discolored hypocotyls were assigned to the early wilt category. Wilting and chlorotic plants were included in the late wilt phase. All surviving plants were examined for internal vascular discoloration. Fifteen days after transplanting, data were recorded for each disease category. The results are presented in Table 10.

Table 10.--Disease incidence in 2-week old soybean plants that were transplanted to Phytophthora-infested loam.

Variety	Percentage of plants with		
	Early wilt	Late wilt	Total diseased[a]
Monroe	0.0	2.4	2.4
Earlyana	47.6	52.4	100.0
W9-2024	100.0	0.0	100.0

[a] No diseased plants in checks; 7 replicates of 6 transplants each.

The differences in resistance in the early wilt and total disease categories between Monroe and Earlyana or strain W9-2024 were highly significant. Earlyana was significantly more susceptible in the late wilt phase of the disease than either of the other 2 varieties. Earlyana was significantly more resistant in the early wilt phase than soybean strain W9-2024. Two days after

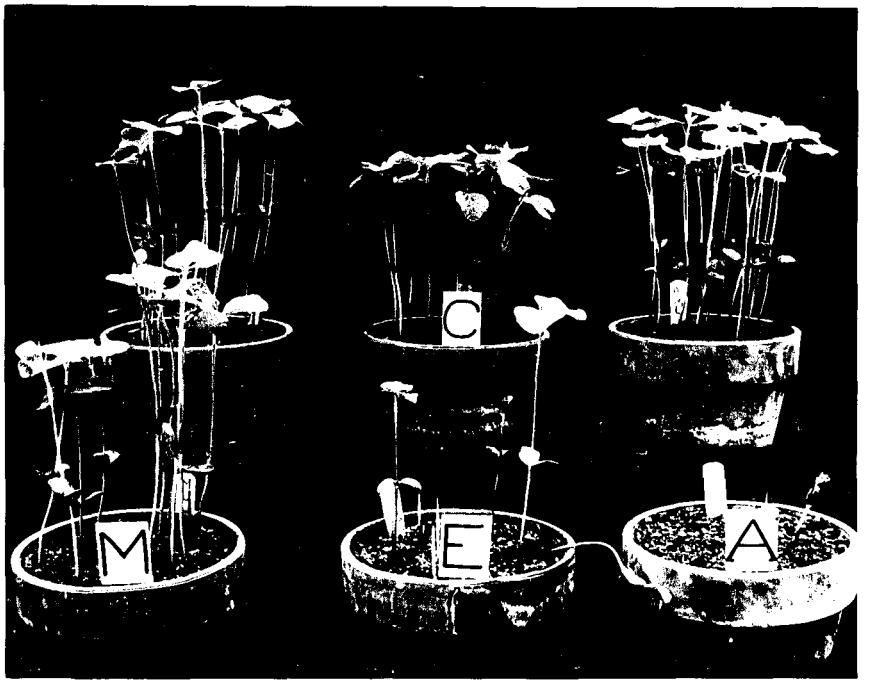

Figure 16.--Two-week old soybean plants in Toledo silty clay. Back row, non-infested soil; front row, Phytophthora-infested soil. Monroe (M), Earlyana (E), and W9-2024 (A).

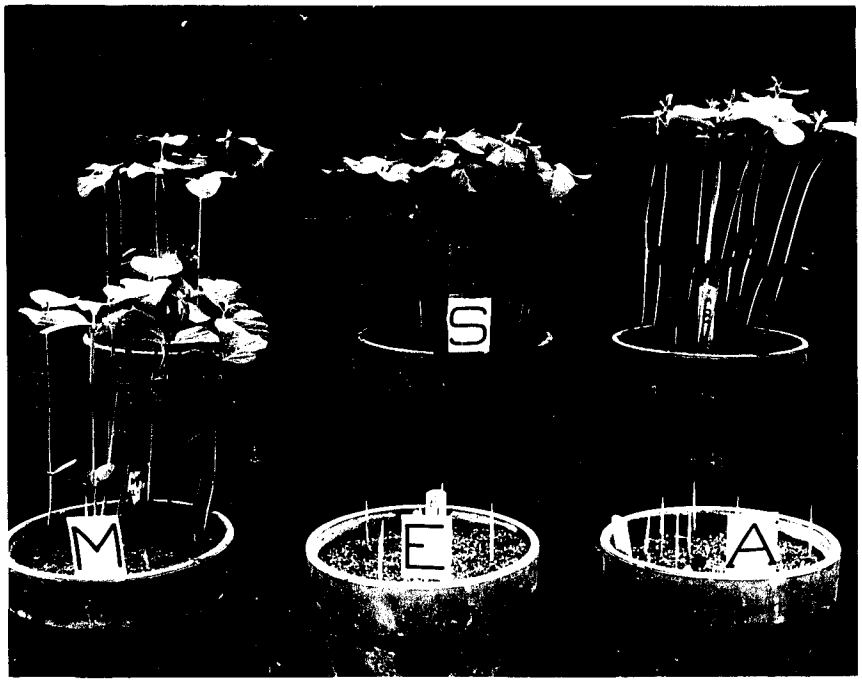

Figure 17.--Two-week old soybean plants in sand. Back row, non-infested soil; front row, Phytophthora-infested soil. Monroe (M), Earlyana (E), and W9-2024(A).

being transplanted to infested soil, the unifoliate leaves of soybean strain W9-2024 were flaccid and some marginal chlorosis was evident. After 6 days, the wilted and shriveled leaves became brittle and necrotic. Symptom development in Earlyana was not as rapid and as severe as in strain W9-2024, but the plants were markedly stunted. Monroe soybean plants in the Phytophthora-infested soil were as vigorous and healthy as the checks.

Soil temperatures

It was inferred from the field results that some soybean varieties and strains were most severely affected after the later planting dates when the soil temperatures were higher. Since the pathogenicity of the Phytophthora species was tested only at a soil temperature of 26°-27° C. in previous experiments, the soil temperature relations to disease incidence was investigated.

The soil temperatures were maintained at 15°, 25°, and 35° C. respectively, in Wisconsin-type temperature tanks. Each variety-treatment was replicated 5 times and pots of plants in steamed, non-infested soil served as checks. The results are presented in Table 11. These data were analyzed statistically and the differences in susceptibility of the varieties at the 3 soil temperatures were significant. Orthogonal comparisons of the individual treatments of the data are summarized in Table 12.

Monroe was significantly more resistant than either Earlyana or strain W9-2024 at soil temperatures of 15° and 25° C. At 35° C., no significant difference occurred in susceptibility of

Table 11.--Pre-emergence damping-off (Pre), post-emergence damping-off (Post), and total disease incidence (Total) in soybean plants after 3 weeks in Phytophthora-infested Wooster silty loam maintained at three temperatures.

	Per cent of plants diseased								
	15° C.			25° C.			35° C.		
Variety	Pre	Post	Total	Pre	Post	Total	Pre	Post	Total
Monroe	7.3	5.6	12.9	4.0	2.4	6.4	22.4	73.6	96.0
Earlyana	32.3	49.2	81.5	54.0	41.9	95.9	67.8	31.4	99.2
W9-2024	41.9	44.4	86.3	53.7	44.7	98.4	58.1	41.1	99.2

Table 12.--Significances of one-degree-of-freedom comparisons between the treatments of the data of Table 11.

	15° C.			25° C.			35° C.		
Treatment	Pre	Post	Total	Pre	Post	Total	Pre	Post	Total
Mon.-Early.	5[a]	1	1	1	1	1	1	1	ns
Mon.-W9	1	1	1	1	1	1	1	5	ns
Early.-W9	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Monroe			Earlyana			W9-2024		
15°-25° C.	ns	ns	ns	ns	ns	1	ns	ns	ns
15°-35° C.	5	1	1	1	ns	1	ns	ns	5
25°-35° C.	5	1	1	ns	ns	ns	ns	ns	ns

[a] 5-5 per cent level of significance
1-1 per cent level of significance
ns - not significant

Monroe from the other 2 varieties. The difference in susceptibility between Earlyana and strain W9-2024 at the 3 temperatures was not significant.

The difference in susceptibility of Monroe at 35° C. and the other temperatures was highly significant. Earlyana was significantly more susceptible at 35° C. and 25° C. than at the 15° C. soil temperature. Soybean strain W9-2024 was significantly more susceptible at 35° than at 15° C.

Soybean varieties Blackhawk, Lincoln, and Harosoy, were used in a similar experiment on the effect of soil temperatures on the pathogenicity of the Phytophthora species. No check plants were diseased. The results are presented in Table 13. Orthogonal comparisons of the individual treatments of the data are presented in Table 14. Blackhawk was significantly more resistant than either Lincoln or Harosoy at the 3 soil temperatures. The differences in susceptibility between Lincoln and Harosoy at all temperatures were not significant.

Blackhawk was significantly more susceptible at 35° than at either 15° or 25° C. Both Lincoln and Harosoy were significantly more susceptible at 25° and 35° than at 15° C. At 35° C., a high incidence of post-emergence damping-off occurred in Blackhawk and Monroe.

Table 13.--Pre-emergence damping-off (Pre), post-emergence damping-off (Post), and total disease incidence (Total) in soybean plants after 3 weeks in Phytophthora-infested Wooster silty loam maintained at three temperatures.

	Per cent of plants diseased								
	15° C.			25° C.			35° C.		
Variety	Pre	Post	Total	Pre	Post	Total	Pre	Post	Total
Blackhawk	4.9	0.0	4.9	5.6	3.2	8.8	16.1	61.3	77.4
Lincoln	39.7	54.5	94.2	40.2	59.0	99.2	33.6	64.0	97.6
Harosoy	20.8	68.8	89.6	22.0	76.4	98.4	9.8	89.3	99.1

Table 14.--Significances of one-degree-of-freedom comparisons between the treatments of the data of Table 13.

Treatment	15° C.			25° C.			35° C.		
	Pre	Post	Total	Pre	Post	Total	Pre	Post	Total
Black.-Lin.	1[a]	1	1	1	1	1	1	ns	1
Black.-Har.	5	1	1	ns	1	1	ns	1	1
Lin.-Har.	5	5	ns	5	5	ns	1	1	ns
	Blackhawk			Lincoln			Harosoy		
15°-25° C.	ns	ns	ns	ns	ns	5	ns	ns	5
15°-35° C.	5	1	1	ns	ns	5	ns	5	5
25°-35° C.	5	1	1	ns	ns	ns	ns	ns	ns

[a] 1-1 per cent level of significance
5-5 per cent level of significance
ns - not significant

Effect of other fungi

A representative species of Fusarium, frequently isolated prior to the isolation of the pathogenic Phytophthora, was used to study the effect of other fungi on the pathogenicity of this organism. Loam was infested with the Fusarium species alone, with the Phytophthora species alone, and with a mixture of the 2 fungi. The fungi were increased in potato-dextrose broth for 2 weeks, and 100 ml of the inoculum were added to each 6-inch pot of loam. Each treatment was replicated 4 times. The results are presented in Table 15.

The differences between treatments were significant. No symptoms occurred in plants growing in soil infested with the Fusarium species. Significantly less disease incidence occurred in plants in soil infested with a combination of the fungi than in soil infested with Phytophthora only. The difference in

Table 15.--Disease incidence in Earlyana soybean plants after 3 weeks in soil infested with: Phytophthora, Fusarium, and a combination of these 2 fungi.

Treatment	Per cent of diseased plants		
	Pre-emergence damping-off	Post-emergence damping-off	Total disease
Phytophthora	59.2	40.8	100.0
Fusarium	0.0	0.0	0.0
Phytophthora and Fusarium	14.3	62.2	76.5

susceptibility of soybean plants in the soil infested with both fungi and in Phytophthora-infested loam was highly significant. The difference in resistance of plants to post-emergence damping-off in soil infested with the combination of fungi and in Phytophthora-infested soil was not significant.

The effect of other organisms on the virulence of the Phytophthora species was investigated. Soil was obtained from a field in which soybeans had been cultivated for 2 consecutive years, but in which no root rot appeared. One portion of the soil was autoclaved: half of it was infested with Phytophthora. The other portion of soil was not autoclaved but half of it was infested. Twenty-five Harosoy soybean seeds were planted in each 6-inch pot of soil. The results are presented in Table 16. The difference in severity of the disease in steamed, Phytophthora-infested soil and in non-steamed, infested soil was highly significant.

Table 16.--Disease incidence in Harosoy soybean plants after 3 weeks in Phytophthora-infested, steamed and non-steamed soil.

Treatment	Percentage of diseased plants[a]		
	Pre-emergence damping-off	Post-emergence damping-off	Total disease
Non-steamed, infested soil	14.1	47.5	61.6
Non-steamed, non-infested soil	0.0	0.0	0.0
Steamed, infested soil	18.2	76.8	95.0

[a] No diseased plants in checks (steamed, non-infested soil).

Inoculum concentration

The inoculum, used throughout this work, consisted of 100 ml of 2-week old culture in potato-dextrose broth per each 6-inch pot of soil. To study the effect of time on post-emergence disease incidence, using various concentrations of inoculum, the stock inoculum (X) was diluted 1/10 , 1/100, 1/1000, and 1/10,000, respectively with distilled water. Hereafter; 1/10 equals X/10, 1/100 equals X/100, 1/1000 equals X/1000, and 1/10,000 equals X/10,000. At 1-week intervals for 3 weeks, data on disease incidence was recorded. Most of the seedlings in the non-infested soil emerged within the first week. The results are presented in Figure 18. No disease symptoms were evident in plants growing in soil infested with X/1000 and X/10,000 concentrations of inoculum. At X, X/10, and X/100 concentrations, disease incidence varied directly with time. The root rot was more severe at the X concentration than at the X/10 and X/100 levels.

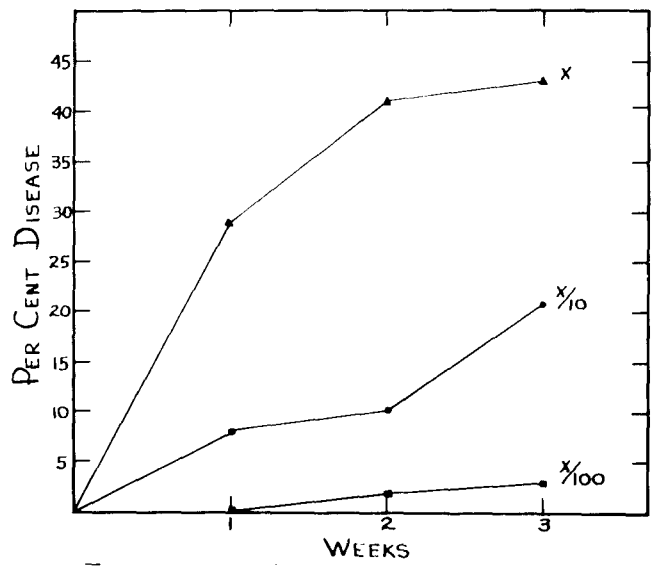

Figure 18.--Post-emergence disease incidence in Lincoln soybean plants at 1, 2, and 3-week intervals in soil infested with various concentrations of Phytophthora inoculum.

At the X concentration, the disease incidence increased markedly during the first and second week but tended to level-off after 2 weeks. Such limiting factors as the number of plants, antagonism of soil microorganisms, and the accumulation of toxic substances may be operating at this time.

Disease incidence increased gradually during the first and second weeks at the X/10 concentration. After the second week, the disease incidence increased sharply. This increase was of the approximate magnitude as that which occurred at the X concentration.

At the X/100 concentration, no disease developed until after the first week. Thereafter, the disease incidence increased very slightly.

The effect of various dilutions of Phytophthora inoculum on disease incidence in Lincoln soybean plants after 3 weeks was studied. Percentage of diseased plants was calculated on the basis of the number of seeds planted. No diseased plants were found in the checks. The results are illustrated in Figure 19, and the data are summarized in Table 17.

Table 17.--Disease incidence in Lincoln soybean plants after 3 weeks in soil infested with various inoculum concentrations of Phytophthora.

Concentrations	Percentage of diseased plants		
	Pre-emergence damping-off	Post-emergence damping-off	Total disease
X	39.2	54.0	93.2
X/10	19.2	31.0	50.2
X/100	0.8	3.0	3.8
X/1000	0.0	0.0	0.0
X/10,000	0.0	0.0	0.0

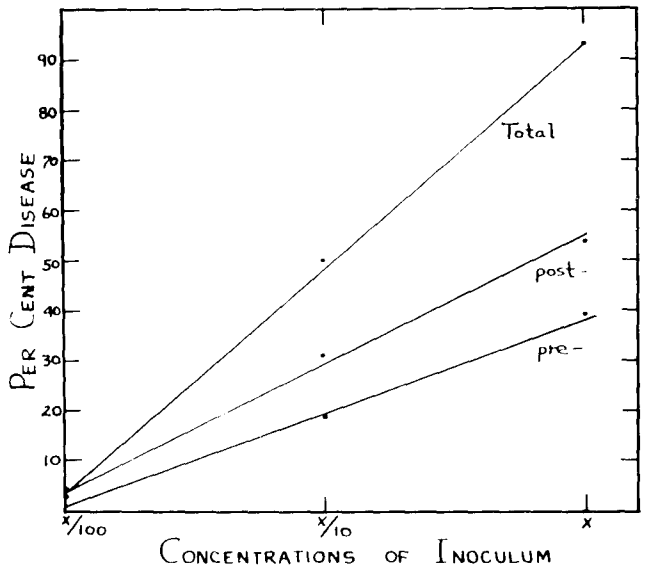

Figure 19.--Disease incidence of Lincoln soybean plants after 3 weeks in soil infested with different concentrations of Phytophthora inoculum.

The differences between the treatments were significant. Significantly more disease was evident at the X concentration than at all other concentrations. The differences in disease incidence between X/10 and all lower concentrations were highly significant. The differences in the per cent of disease at the X/100 and the other 2 lower concentrations were not significant.

Under the conditions of this experiment, the disease incidence was a function of the logarithm of the concentration of Phytophthora inoculum in the soil (see Figure 19).

Control Measures

The use of resistant varieties, chemical seed-treatments, addition of soil amendments, and crop rotation are 4 possible methods of controlling soil-borne plant pathogens. The investigations herein were confined to these measures and only indirect evidence was obtained for the latter measure.

Soybean varietal tests

Four soybean varieties are recommended for Ohio (5). These are Monroe, Lincoln, Hawkeye, and Harosoy.

Monroe is confined to the northern part of the state where earliness is a prime factor.

Lincoln, the latest maturing variety recommended for Ohio, is confined to the southern half of the state. The high yield and high oil content of this variety have never been surpassed in the state.

Hawkeye, 8 days earlier than Lincoln and 10 days later than Monroe, is the best variety in maturity Group II in yield and oil content.

Harosoy, released in 1952, is 3 days earlier than Hawkeye and is more resistant to stem canker.

Although the varieties are not on the recommended list, large acreages of Blackhawk, Bavender, Earlyana, and Adams are still planted in Ohio.

OH-53 is a selection from Bavender, made by the writer at Oak Harbor, Ohio, in 1953. This site was the one chosen for the disease nursery in 1954. The selection was made in a field of soybeans

in which practically every plant examined, except 2, had symptoms of the root rot. The seeds of these plants were processed and saved for further evaluation. Because of the performance of OH-53 in the nursery and in preliminary greenhouse tests, it warranted further study.

OH-53 is a member of maturity Group II, has gray pubescence, purple flowers, 3- and 4-seeded pods, and yellow seeds with light-brown hila.

The L.S.D. (least significant difference) between the variety means for susceptibility to the Phytophthora root rot was determined by the analysis of variance test. The results are summarized in Table 18.

Table 18.--Mean number of soybean plants in Phytophthora-infested loam after 3 weeks[a].

Variety	Killed before emergence	Killed after emergence	Total diseased
OH-53	1.05	0.25	1.30
Monroe	1.55	0.50	2.05
Blackhawk	4.30	2.75	7.05
Harosoy	4.50	19.00	23.50
Adams	9.50	14.50	24.00
Hawkeye	5.75	19.00	24.75
Lincoln	11.50	13.50	25.00
L.S.D. 1%	5.00	4.92	2.66
L.S.D. 5%	3.68	3.62	1.96

[a] Mean of 4 replications of 25 seeds each; no diseased plants in checks.

Soybean strain OH-53, Monroe, and Blackhawk were significantly more resistant than Harosoy, Adams, Hawkeye, or Lincoln. The differences in susceptibility between Monroe and Blackhawk and between OH-53 and Blackhawk were highly significant.

No significant difference in resistance existed between OH-53 and Monroe. The differences in susceptibility between Harosoy, Adams, Hawkeye, and Lincoln were not significant. Lincoln and Adams were significantly more susceptible to pre-emergence damping-off than the other soybean varieties. Soybean strain OH-53, Monroe, and Blackhawk were significantly more resistant to post-emergence damping-off than the other varieties. The differences between OH-53, Monroe, and Blackhawk in susceptibility to pre- or post-emergence damping-off were not significant.

Bavender seed was not available for this test. Subsequently, seed was obtained and the susceptibility to the Phytophthora species of Bavender and OH-53 was compared. The results are presented in Table 19. Strain OH-53 was significantly more resistant than Bavender (Figure 20).

Table 19.--Disease incidence in soybean plants after 3 weeks in Phytophthora-infested loam.

Variety	Percentage of diseased plants		
	Pre-emergence damping-off	Post-emergence damping-off	Total disease
Bavender	59.0	32.0	91.0
OH-53	12.5	3.0	15.5

Seed treatments

It was thought that treating soybean seeds with commercial fungicides might control pre-emergence damping-off and seed decay. The seed-treatment compounds and their composition are listed in Table 20. All these materials were applied at the rate of 2 oz. per bushel. In the checks, in which the treated-seeds were planted

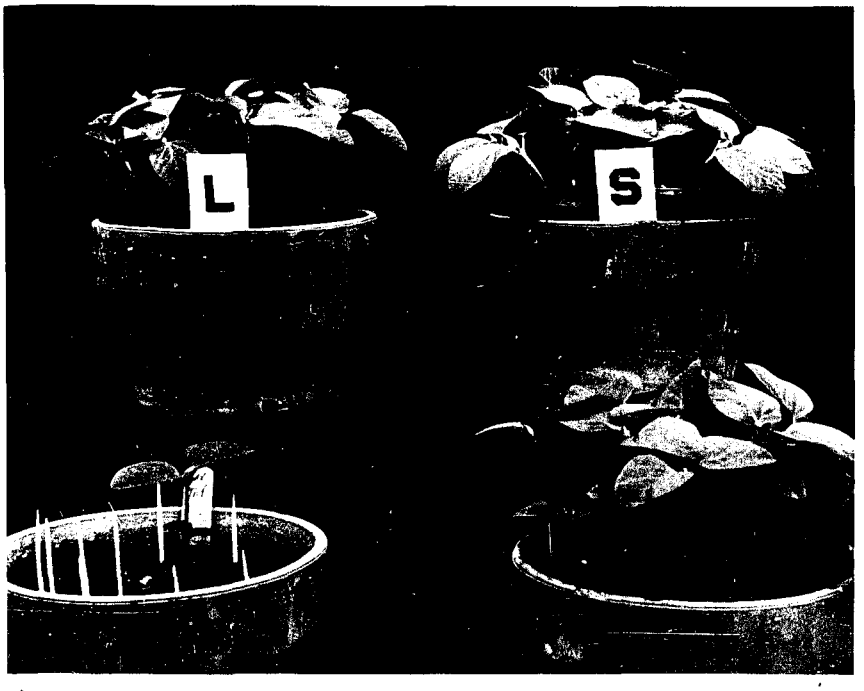

Figure 20.--Eleven-day old soybean plants in non-infested soil (top row), and Phytophthora-infested soil (bottom row). Bavender (L), and OH-53(S).

in non-infested, steamed soil, the plants were healthy and vigorous.

Table 20.--Fungicides used in the treatment of soybean seeds planted in Phytophthora-infested soils.

Trade Name	Coined Name and Per Cent of Active Material	Active Material
Orthocide 75 Seed Protectant	captan, 75.0	N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide
Arasan	thiram, 50.0	tetramethylthiuram disulfide
Spergon	chloranil, 98.0	tetrachloro-p-benzoquinone
Phygon	dichlone, 97.0	2,3-dichloro-1,4-naphthoquinone
Ceresan M	7.7	ethyl mercury p-toluene sulfonanilide

These data were subjected to the analysis of variance test, in which the check was considered as a treatment. The differences in disease incidence between treatments and the check were not significant. Some of the differences between the treatments were statistically significant, however. The data of the seed-treatment experiments are presented in Tables 21 and 22. The results are illustrated in Figures 21 and 22.

Ceresan M, applied at the rate of 2 oz. per bushel, was phytotoxic to soybean seedlings. After 3 weeks, most plants from Ceresan M-treated seed were only in the cotyledonary stage whereas a few were of normal size. These seedlings were swollen considerably at the hypocotyls and basal portion of the stems. Thiram-and captan-treated seeds were significantly less susceptible

Table 21.--Disease incidence and seedling emergence in Harosoy soybean plants after 3 weeks from seed treated with fungicides and planted in Phytophthora-infested loam.

Treatment	Percentage of diseased plants[a]			Per cent emergence
	Pre-emergence damping-off	Post-emergence damping-off	Total disease	
captan	6.0	77.0	83.0	100.0
thiram	7.0	76.0	83.0	100.0
chloranil	10.0	78.0	88.0	100.0
Ceresan M	24.2	71.0	95.2	97.0
None	15.2	76.0	91.2	99.0

[a] No diseased plants in checks; 4 replicates of 25 seeds each.

Table 22.--Disease incidence and seedling emergence in Earlyana soybean plants after 3 weeks from seed treated with fungicides and planted in Phytophthora-infested Toledo silty clay.

Treatment	Percentage of diseased plants[a]			Per cent emergence
	Pre-emergence damping-off	Post-emergence damping-off	Total disease	
dichlone	47.2	50.0	97.2	97.0
thiram	9.2	85.0	94.2	99.0
chloranil	19.2	75.0	94.2	98.0
captan	8.2	84.0	92.2	100.0
None	29.2	67.0	96.2	99.0

[a] No diseased plants in checks; 4 replicates of 25 seeds each.

to pre-emergence damping-off than those treated with Ceresan M. Less pre-emergence damping-off occurred from the captan-and thiram-treated Harosoy seed than from non-treated seed, but most of these seedlings were affected later. This was also true for the same 2 compounds on Earlyana seed planted in Phytophthora-infested Toledo silty clay.

Plants from dichlone-treated seeds were significantly more susceptible to pre- and post-emergence damping-off than those

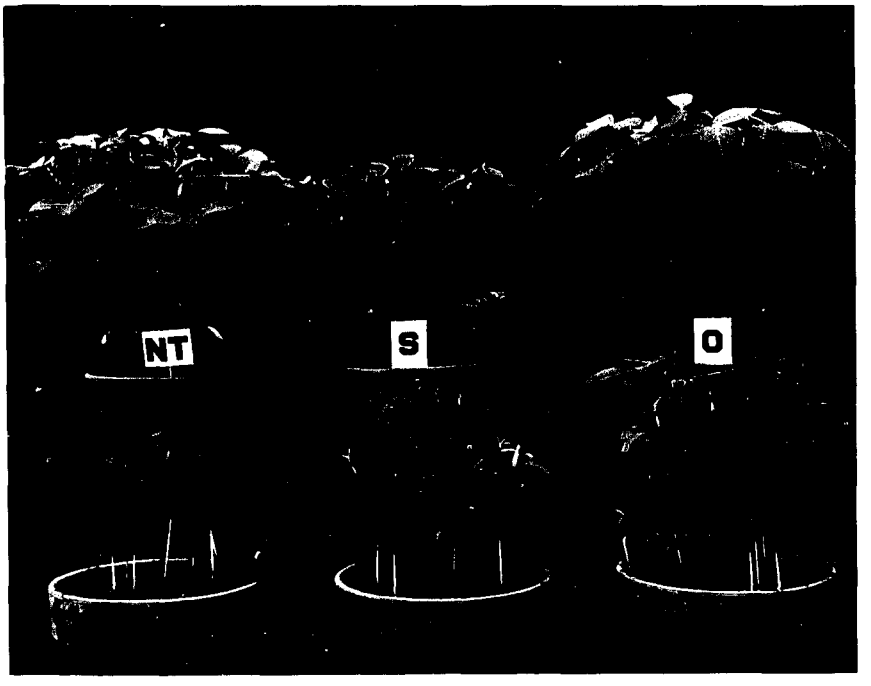

Figure 21.--Twenty-two day old soybean plants from chemically-treated seed. Top row, non-infested soil; bottom row, Phytophthora-infested soil. No treatment (NT), chloranil (S), and captan (O).

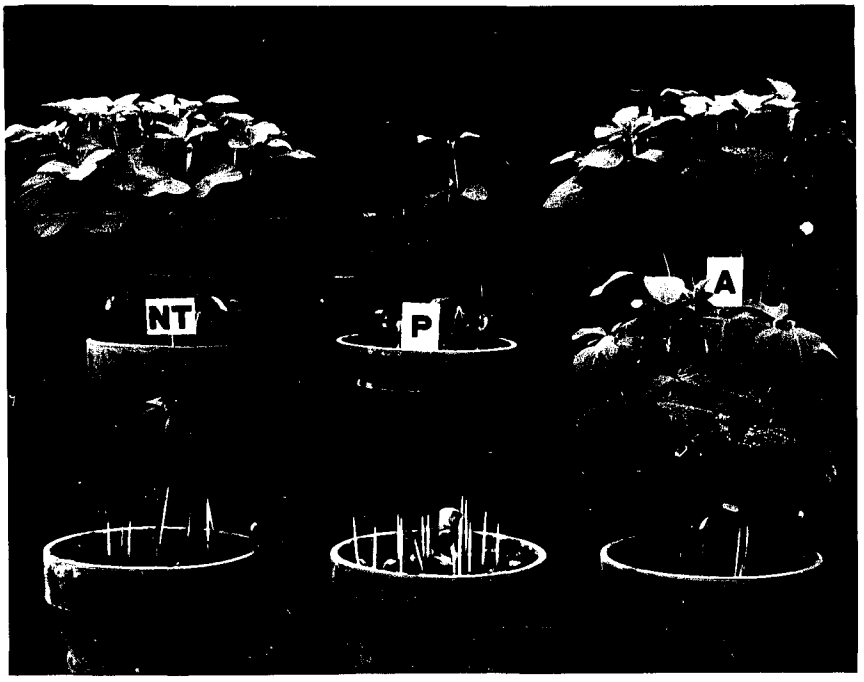

Figure 22.--Twenty-two day old soybean plants from chemically-treated seed. Top row, non-infested soil; bottom row, Phytophthora-infested soil. No treatment (NT), Ceresan M (P), and thiram (A). Note phytotoxicity of Ceresan M (P).

from seeds treated with either captan or thiram. Soybean plants from chloranil-treated seeds in non-infested steamed soil were slightly stunted (Figure 21). Captan was significantly more effective than dichlone. The seedling emergence from treated-soybean seeds in non-infested soil was virtually 100 per cent.

Fertilizer applications

The effect of fertilizer on the disease incidence of Lincoln soybeans was studied. 0-20-20 fertilizer was applied to Toledo silty clay at the rates of 200 and 400 pounds per acre, respectively. One hundred ml of a 2-week old Phytophthora culture in potato-dextrose broth were added to each 6-inch pot of soil. The checks consisted of non-infested soil to which fertilizer was applied at the same rates. The data are presented in Table 23 and the results are illustrated in Figure 23.

Table 23.--Disease incidence and seedling emergence of Lincoln soybean plants after 3 weeks in Phytophthora-infested Toledo silty clay to which 0-20-20 fertilizer was added.

Treatment	Percentage of diseased plants			Per cent emergence
	Pre-emergence damping-off	Post-emergence damping-off	Total disease	
Fertilizer-200 lbs/acre	34.2	65.0	99.2	99.0
Fertilizer-400 lbs/acre	52.0	48.0	100.0	100.0
Check	51.0	47.0	98.0	98.0

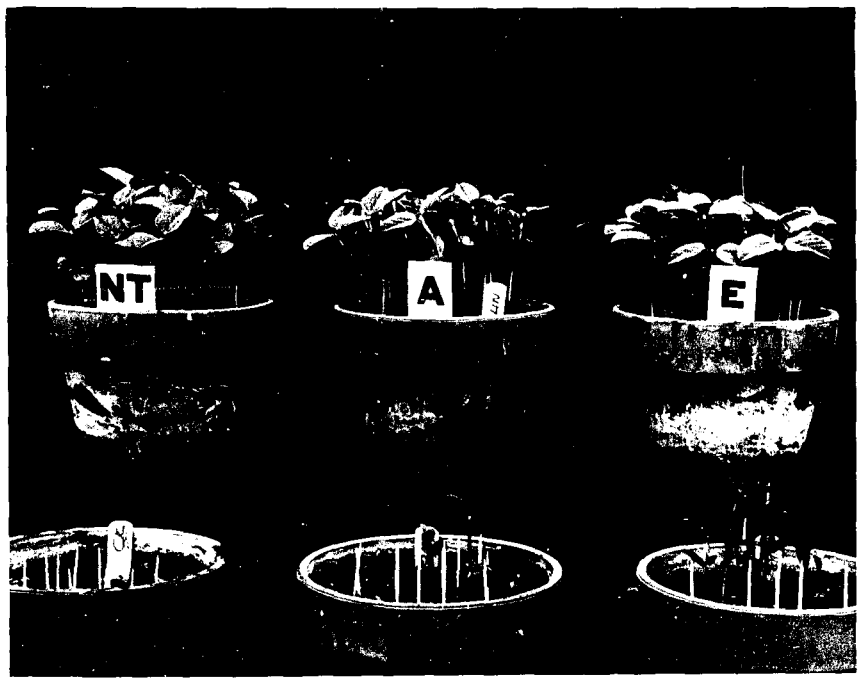

Figure 23.--Ten-day old Lincoln soybean plants in soil to which 0-20-20 fertilizer was added. Top row, non-infested soil; bottom row, Phytophthora-infested soil. No fertilizer (NT), 200 pounds/acre (A), and 400 pounds/acre (E).

The differences between the fertilizer treatments and the check, in regard to total disease incidence, were not significant. Significantly more post-emergence damping-off occurred at the 200-pound-per-acre fertilizer treatment than in the check. At the 400-pound-per-acre application, significantly more pre-emergence damping-off was evident than at the 200-pound-per-acre treatment. Seedling emergence was virtually 100 per cent in all treatments.

Suspect range

A number of plant species were tested for susceptibility to the Phytophthora species. Varieties which are commonly cultivated in Northwestern Ohio were used. As a check on the viability of the inoculum, Earlyana soybean seeds were planted in infested soil. Checks consisted of seeds planted in steamed, non-infested soil. The results are summarized in Table 24. These data were based on the results of 2 replications of 50 seeds each.

Table 24.--Per cent germination of various crops in steamed and in Phytophthora-infested loam after 3 weeks.

Crop	Variety	Germination-Per cent of seeds planted	
		Steamed soil	Infested soil
Oats	Clinton 59	100.0	99.0
Timothy		100.0	99.0
Alfalfa	Ranger	99.0	98.0
Red clover	Kenland	99.0	100.0
Corn	Ohio C54	98.0	99.0
Wheat	Thorne	97.0	98.0
Soybeans	Earlyana	97.0	0.0

DISCUSSION

The Phytophthora root rot of soybeans occurs in Northwestern Ohio in the old lake-bed soils such as Brookston, Clyde, and Toledo silty clays. In such soils, the disease is usually more severe in poorly drained parts of the field. The high water-retaining capacity of clay soils may be more suited for the survival and increase of the organism than lighter soil types. The relationship between soil-water and pathogenicity of the fungus needs to be investigated. In the greenhouse, severe disease incidence was evidenced in soybean plants of susceptible varieties in Phytophthora-infested sand and loam. In nature, the distribution and survival of the inoculum and not soil types may be limiting factors in the distribution of this root rot of soybeans.

The rate and total amount of radial growth of the Phytophthora species varied considerably on different media and at various temperatures. In describing or identifying an organism therefore, it is necessary to specify the medium upon which the fungus is cultured.

The optimum pH for growth in diameter of the Phytophthora colonies on buffered corn meal agar was 9 which is unusual as most of the pH optima reported for many fungi are less than 7 (15). A secondary optimum occurred at approximately pH 6.3. The secondary peaks for many fungi occur at, or near, the neutral point (15). The pH values in the alkaline series were slightly lower than the initial values. This decrease in alkalinity may

be explained on the basis of the production of CO_2 or organic substances as a result of the metabolic activities of the fungus. The physiology of the organism in regard to the possible production of substances toxic to the host should be studied.

Using the monograph of Tucker (24), the species of this fungus was not satisfactorily identified. The species of this Phytophthora warrants identification.

Two resistant soybean varieties of maturity Group II, Monroe and Blackhawk, were significantly more susceptible at a soil temperature of 35° than at either 15° or 25° C. The larger portion of the disease incidence in both varieties at 35° C. was post-emergence damping-off. The nature and mechanism of susceptibility of these 2 varieties at a soil temperature of 35° C. warrants investigation. The susceptibility of Blackhawk and Monroe at 35° C. may not be highly significant from a practical standpoint as relatively low soil temperatures usually prevail during the early spring when most of the soybean acreage is planted in Ohio. Furthermore, no effect of the 4 planting dates on disease incidence was evident from the field results. Monroe and Blackhawk were resistant under field conditions even when seeded as late as June 22.

Evidence that variations in the quantity of Phytophthora inoculum influenced disease incidence in soybean plants in steamed, infested soil, was obtained. The incidence of disease was a function of the logarithm of fungus concentration in the soil.

In any root rotting disease, 4 possible means of control are available. First, the causal agent may be eliminated from the soil in the immediate vicinity of the host. This may be accomplished by seed-treatment chemicals, or the soil may be treated directly with fungicidal agents. Second, varieties and strains of the host plant may be found or developed that are resistant to soil-borne pathogens. Third, soil amendments such as fertilizers or lime may change the physico-chemical nature of the soil. This change, in turn, affects the development of the disease. The fourth approach is related to the third measure and involves a change in the soil microflora with a resultant biological control of the causal organisms by non-pathogenic soil organisms. Such practices as crop rotation and plowing-under of a green manure crop are included in this measure.

Soybean seeds treated with captan, dichlone, thiram, chloranil, and Ceresan M were planted in soil, heavily infested with the Phytophthora species. These chemicals were applied at the rate of 2 oz. per bushel. The differences between the treatments and checks were not significant. The per cent germination of thiram- and captan-treated seeds was better than that of non-treated seeds; however, seedlings from the treated-seeds became diseased later in their development. These seed-treatments were at soil temperatures of approximately 26° to 28° C. It is possible that some of these treatments are effective at lower soil temperatures. A higher rate of application may be needed for protection of the soybean seeds during germination in Phytophthora-infested

soils. The effectiveness of seed-treatments in the field, where the relative pathogenicity of the Phytophthora species is less, warrants investigation.

Of the 4 soybean varieties recommended for Ohio; Harosoy, Hawkeye, Lincoln, and Monroe, only the latter is resistant to the Phytophthora species inciting a root rot of soybeans. Although the varieties are not on the recommended list, large acreages of Blackhawk, Bavender, Earlyana, and Adams are still planted in Ohio. Of this group, only Blackhawk is resistant. The varieties Monroe and Blackhawk should be used where this root rot of soybeans is severe.

Of the 95 soybean varieties and strains tested in the disease nursery, only a few were resistant. A disease index of 1 was assigned to those strains and varieties in which the disease incidence ranged from 0 to 20 per cent. In 3 of the 5 maturity groups, no indices of 1 were assigned. In such instances, the strains and varieties with the lowest per cent total disease were considered the best in their respective maturity group.

Since the Phytophthora species did not grow well on various types of solid media at lower pH values of 3 to 5, the effect of 0-20-20 fertilizer (pH, approximately 4) on the pathogenicity of the fungus was investigated. The differences in susceptibility of the soybean plants to the Phytophthora species between treatments and the check were not significant.

Less disease incidence was evident in soybean plants growing in soil infested with a combination of Phytophthora and

Fusarium than in soil infested with the Phytophthora species only. Apparently, the non-pathogenic Fusarium species, in association with the pathogenic species of Phytophthora, results in less disease incidence. This effect evidently occurs at the early stages of disease development as evidenced by the severity of pre-emergence damping-off.

It is suggested that the pathogenic Phytophthora species invades the soybean seedlings and establishes a host-parasitic relationship. Soon thereafter, secondary organisms, especially Fusarium species, enter via the avenues of entrance already established. In the post-emergence damping-off phase of the disease, the symptoms are characteristic of those incited by wilt-type pathogens. The secondary organisms become so readily established in the host tissue that it is difficult to isolate the pathogenic Phytophthora species from diseased soybean plants older than 2 weeks.

More evidence of the decrease of pathogenicity of the Phytophthora species, when in association with other fungi, was obtained. Soil was collected from a field in which no root rot occurred and a portion of it was autoclaved; the remainder was not steamed. Both lots of soil were infested with the Phytophthora species. A higher disease incidence was evidenced in the Harosoy plants in steamed, infested soil than in the plants growing in non-steamed, infested soil. This effect may be the explanation for the difference in the degree of virulence of the Phytophthora species under field and greenhouse conditions.

Oats, wheat, corn, timothy, red clover, and alfalfa were not susceptible to the Phytophthora species. Since the fungus is not parasitic to these crops, it is suggested that the organism cannot survive and increase on these plants. Any of these crops can be employed where this disease of soybeans is a serious problem.

Thus far, this Phytophthora root rot of soybeans has been found only in clay and silty clay soils in Northwestern Ohio. However, factors such as weather favorable to disease development, continual cropping of soybeans in the same field, and the presence of large acreages of susceptible varieties, could result in a serious epidemic.

SUMMARY

In 1953 and 1954, a destructive root rot of soybeans was found in Northwestern Ohio. In 1954, a species of Phytophthora was isolated from diseased, 2-week old soybean seedlings after many non-pathogenic Fusarium species had been frequently isolated previously. The cultural characteristics as well as the pathogenicity on Bavender soybeans of the Phytophthora isolates were similar. One isolate was selected at random and used throughout the investigation.

Symptoms of the disease may appear on soybean plants from the unifoliate-leaf stage to the pod-forming stage. Pre-emergence damping-off and seed decay, post-emergence damping-off, and root rotting are 3 phases of the disease. In the latter phase, stunting, wilting, and chlorosis are prominent external symptoms. Discoloration of the vascular tissues, and an extensive decay of the root system are evident.

In 1954, Toledo silty clay was obtained from fields in which the disease occurred and a part of it was steamed. Soybean plants in the non-steamed soil were diseased whereas no diseased plants were evident in the steamed soil.

The effect of temperature and media on radial growth of the Phytophthora species, over a range of temperatures from 6° to 35° C., was studied. The growth of the fungus was best on yellow corn meal agar. The cardinal temperatures on this medium were: less than 6°, 26°, and greater than 35° C., respectively.

On potato-dextrose, malt, and yeast extract agars, the cardinal temperatures were: 10°, 22°, and 30° C., respectively. The respective cardinal temperatures on nutrient agar were: less than 6°, 24°, and 30° C.

The effect of hydrogen-ion concentration on radial growth of the Phytophthora species was investigated. The optimum pH for growth of this fungus on buffered corn meal agar was 9. A secondary optimum occurred at approximately pH 6.3.

The Phytophthora root rot of soybeans has been found in Ohio only in the old lake-bed soils such as Brookston, Clyde, and Toledo silty clays. In such soils, the disease is usually more severe in poorly drained parts of the field.

In the greenhouse, Earlyana was significantly more susceptible in Toledo silty clay than in loam, whereas strain W9-2024 was more susceptible in clay than in either loam or sand. Monroe was significantly more susceptible in loam and sand than in Toledo silty clay. Monroe was more resistant than either Earlyana or strain W9-2024 in clay, loam, and sand.

The relationship of the age of soybean plants and susceptibility to the Phytophthora species was studied. Two-week old plants of varieties Monroe and Earlyana and strain W9-2024 were transplanted to Phytophthora-infested soil. Monroe was significantly more resistant than either of the other varieties. The rate of symptom development was more rapid in soybean strain W9-2024 than in Earlyana. The difference in susceptibility between Earlyana and W9-2024 was not significant.

The effect of soil temperatures on the pathogenicity of the Phytophthora species was investigated. Monroe was significantly more resistant than either Earlyana or strain W9-2024 at soil temperatures of 15° and 25° C. The differences in susceptibility of Monroe from the other 2 varieties at 35° C. were not significant. Monroe and Blackhawk were significantly more susceptible at 35° than at soil temperatures of 15° and 25° C. Blackhawk was more resistant than either Lincoln or Harosoy at soil temperatures of 15°, 25°, and 35° C., respectively.

The relationship of inoculum concentration and disease incidence of soybean plants in Phytophthora-infested soil was investigated. The per cent of diseased plants in infested soil was a function of the concentration of inoculum.

The effect of other organisms on pathogenicity of the Phytophthora species was evident in a test in which soil from a soybean field, in which no root rot occurred, was used. The fungus was more virulent on soybean plants in steamed, Phytophthora-infested soil than on those plants growing in non-steamed soil, infested with the fungus.

Significantly less disease incidence occurred in soybean plants growing in steamed soil infested with both Phytophthora and Fusarium than in steamed soil infested with Phytophthora only.

Clinton 59 oats, Ranger alfalfa, Kenland red clover, Ohio C54 corn, Thorne wheat, and timothy were not susceptible to the Phytophthora species. A crop rotation program, in which soybeans

do not follow soybeans, should be employed on land where any trace of the disease has appeared.

Perhaps the most practical and economical control is the use of resistant soybean varieties. Hawkeye, Harosoy, Lincoln, and Monroe are the present recommended varieties for Ohio. Only Monroe is resistant to the Phytophthora root rot. Although the variety is not on the recommended list, large acreages of Blackhawk are planted in the state. Monroe and Blackhawk should be used where this root rot of soybeans is severe.

In the field test, soybean strains and varieties with a disease index of 1 were considered resistant. In some maturity groups, strains with an index of 2, but with low percentage of total disease incidence, were considered as potential breeding material. Of the 95 soybean varieties and strains tested in the disease nursery, only a few were resistant.

Blackhawk, Monroe, and C1109 of maturity Group I were resistant to the Phytophthora species.

Illini was the only resistant variety of maturity Group III.

In the Advanced Preliminary group, soybean strains 24338, 22218, 22153, and 24157 were resistant.

Adams and strain H13501 of maturity Group II were moderately resistant to the Phytophthora species with total disease incidences of 24.7 and 27.1 per cent, respectively.

Comet, Renville, and strain WOS-3180 with total disease incidences of 28.1, 30.6, and 33.8 per cent respectively, were considered as moderately resistant members of maturity Group 0.

No soybean strains or varieties of Group IV had a disease index less than 3 (41 to 60 per cent of the plants diseased).

In the State Variety group, no soybean varieties or strains were resistant.

OH-53, a selection from Bavender, made by the writer, was resistant to this root rot of soybeans.

Planting dates which ranged from May 26 to June 22, had no effect on disease incidence of soybeans in the nursery at Oak Harbor, Ohio, in 1954.

No relationship between the pedigree of the soybean varieties and susceptibility to the disease was evident.

Captan dichlone, thiram, chloranil, and Ceresan M at 2 oz. per bushel were ineffective when treated-soybean seeds were planted in soil infested with Phytophthora.

Applications of 200 and 400 pounds per acre of 0-20-20 fertilizer were not effective in controlling the Phytophthora root rot of soybeans.

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AUTOBIOGRAPHY

I, Albert Joseph Suhovecky, was born on May 3, 1926, at Youngstown, Ohio. I received my primary and secondary education in the public schools of Youngstown. Upon discharge from the Army in January, 1947, I enrolled at Western Reserve University, from which I received the degree Bachelor of Science in February, 1950. From Kent State University, Kent, Ohio, I received the degree Master of Arts in September, 1951. From May to September, 1951, I acted in the capacity of Research Assistant at The Ohio Agricultural Experiment Station while in residence at Kent State University. In October, 1951, I received an appointment as Graduate Assistant at The Ohio State University, where I specialized in Plant Pathology. From January to December, 1952, I was a Research Assistant for The Research Foundation of The Ohio State University. In January, 1953, I was appointed a Research Assistant at The Ohio Agricultural Experiment Station, a position I held while completing the requirements for the degree Doctor of Philosophy.